ELECTROPHORETIC SEPARATION OF PROTEINS

Protein separations in vertical slab gels are performed in a variety of formats. Typically, the separating gel size is in the range of 14 × 14–cm. Smaller formats (6 × 8–cm) are also popular and have been termed minigels. Due to the limited separation area, minigels do not offer the same level of resolution when compared to larger-format gels. However, any procedure performed in the standard format works equally well with minigels, and minigels use less reagent and separate proteins much faster.

One-dimensional gel electrophoresis of proteins can provide information about the molecular size and purity of proteins, as well as the number and molecular size of subunits. By analyzing proteins before and after deglycosylation, it is possible to learn about the carbohydrate content. Information about sulfate, phosphate, and carbohydrate content can be determined for proteins analyzed after various radiolabeling procedures. Furthermore, separated proteins can be recovered from polyacrylamide gels by electroelution (*UNIT 10.5*) for subsequent studies.

The purity and molecular size of a peptide or protein can be assessed by analytical separation in the presence of 0.1% SDS on one-dimensional gels (*UNIT 10.2*) or two-dimensional gels (*UNITS 10.3 & 10.4*). By adding a reducing agent (e.g., 2-mercaptoethanol or dithiothreitol) to the sample, it is possible to determine the number and size of subunits in a pure protein. Nondenaturing gels can be used to examine and isolate the "native" protein.

Two-dimensional gel electrophoresis separates proteins in the first dimension by isoelectric focusing and in the second dimension by electrophoresis in the presence of SDS. By separating proteins in this manner, information is obtained not only about size, as in one-dimensional gels, but also about the charge of a protein. Two-dimensional gels are superior for resolving complex mixtures and for assessing protein purity.

Both one- and two-dimensional gel electrophoreses are high-resolution separation methods that yield protein whose sequence can be determined after either electroelution (*UNIT 10.5*) or electroblotting onto polybrene-coated, derivatized glass fiber sheets or polyvinylidene difluoride (PVDF) membrane filters; all are compatible with gas-phase protein sequencers). In many cases, electrophoretic methods are used after successive modes of conventional types of column chromatography (*UNITS 10.9-10.11*) or HPLC (*UNITS 10.12-10.15*) have been used to increasingly purify a given protein from a crude protein mixture. If the protein is separated under denaturing conditions, any biological activity will likely be lost and, therefore, gel electrophoresis should definitely be the last purification step for a protein whose identity is based on a functional assay.

EXHIBIT F

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